ATGGCCCAAGCCCTGCCCTGGCTCCTGCTGTGGATGGGCGCGGGAGT
GCTGCCTGCCCACGGCACCCAGCACGGCATCCGGCTGCCCCTGCGCA
GCGGCCTGGGGGGCGCCCCCCTGGGGCTGCGGCTGCCCCGGGAGAC
CGACGAAGAGCCCGAGGAGCCCGGCCGGAGGGGCAGCTTTGTGGAGA
TGGTGGACAACCTGAGGGGCAAGTCGGGGCAGGGCTACTACGTGGAG
ATGACCGTGGGCAGCCCCCCGCAGACGCTCAACATCCTGGTGGATACA
GGCAGCAGTAACTTTGCAGTGGGTGCTGCCCCCCACCCCTTCCTGCAT
330 CGCTACTACCAGAGGCAGCTGTCCAGCACATACCGGGACCTCCGGAAG
GGTGTGTATGTGCCCTACACCCAGGGCAAGTGGAAGGGGAGCTGGG
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TTCCCAACCTCTTCTCCCTGCAGCTTTGTGGTGCTGGCTTCCCCCTCAA
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TATCGACCACTCGCTGTACACAGGCAGTCTCTGGTATACACCCATCCGG
CGGGAGTGGTATTATGAGGTGATCATTGTGCGGGTGGAGATCAATGGA
CAGGATCTGAAAATGGACTGCAAGGAGTACAACTATGACAAGAGCATTG
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GGTTTCTGGCTAGGAGAGCAGCTGGTGTGCTGGCAAGCAGGCACCACC
CCTTGGAACATTTTCCCAGTCATCTCACTCTACCTAATGGGTGAGGTTAC
CAACCAGTCCTTCCGCATCACCATCCTTCCGCAGCAATACCTGCGGCCA
GTGGAAGATGTGGCCACGTCCCAAGACGACTGTTACAAGTTTGCCATCT
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CTACGTTGTCTTTGATCGGGCCCGAAAACGAATTGGCTTTGCTGTCAGC
GCTTGCCATGTGCACGATGAGTTCAGGACGGCAGCGGTGGAAGGCCCT
TTTGTCACCTTGGACATGGAAGACTGTGGCTACAACATTCCACAGACAG
ATGAGTCAACCCTCATGACCATAGCCTATGTCATGGCTGCCATCTGCGC
CCTCTTCATGCTGCCACTCTGCCTCATGGTGTGTCAGTGGCGCTGCCTC
CGCTGCCTGCGCCAGCAGCATGATGACTTTGCTGATGACATCTCCCTGC
TGAAG

FIG. 1A

CCATGCCGGCCCCTCACAGCCCCGCCGGGAGCCCGAGCCCGCTGCCCAGGCTGGC
CGCCGCSGTGCCGATGTAGCGGGCTCCGGATCCCAGCCTCTCCCCTGCTCCCGTGC
TCTGCGGATCTCCCCTGACCGCTCTCCACAGCCCGGACCCGGGGGCTGGCCCAGG
GCCCTGCAGGCCCTGGCGTCCTGATGCCCCCAAGCTCCCTCTCCTGAGAAGCCACC
AGCACCACCCAGACTTGGGGGCAGGCGCCAGGGACGGACGTGGGCCAGTGCGAGC
CCAGAGGGCCCGAAGGCCGGGGCCCACCATGGCCCAAGCCCTGCCCTGGCTCCTG
CTGTGGATGGGCGCGGGAGTGCTGCCTGCCCACGGCACCCAGCACGGCATCCGGC
TGCCCCTGCGCAGCGGCCTGGGGGGCGCCCCCCTGGGGCTGCGGCTGCCCCGGG
AGACCGACGAAGAGCCCGAGGAGCCCGGCCGGAGGGGCAGCTTTGTGGAGATGGT
GGACAACCTGAGGGGCAAGTCGGGGCAGGGCTACTACGTGGAGATGACCGTGGGC
AGCCCCCCGCAGACGCTCAACATCCTGGTGGATACAGGCAGCAGTAACTTTGCAGT
GGGTGCTGCCCCCCACCCCTTCCTGCATCGCTACTACCAGAGGCAGCTGTCCAGCA
CATACCGGGACCTCCGGAAGGGTGTGTATGTGCCCTACACCCAGGGCAAGTGGGAA
GGGGAGCTGGGCACCGACCTGGTAAGCATCCCCCATGGCCCCAACGTCACTGTGCG
TGCCAACATTGCTGCCATCACTGAATCAGACAAGTTCTTCATCAACGGCTCCAACTGG
GAAGGCATCCTGGGGCTGGCCTATGCTGAGATTGCCAGGCCTGACGACTCCCTGGA
GCCTTTCTTTGACTCTCTGGTAAAGCAGACCCACGTTCCCAACCTCTTCTCCCTGCAG
CTTTGTGGTGCTGGCTTCCCCCTCAACCAGTCTGAAGTGCTGGCCTCTGTCGGAGG
GAGCATGATCATTGGAGGTATCGACCACTCGCTGTACACAGGCAGTCTCTGGTATAC
ACCCATCCGGCGGGAGTGGTATTATGAGGTGATCATTGTGCGGGTGGAGATCAATG
GACAGGATCTGAAAATGGACTGCAAGGAGTACAACTATGACAAGAGCATTGTGGACA
GTGGCACCACCAACCTTCGTTTGCCCAAGAAAGTGTTTGAAGCTGCAGTCAAATCCA
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CTGGTGTGCTGGCAAGCAGGCACCACCCCTTGGAACATTTTCCCAGTCATCTCACTC
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GTTGTCTTTGATCGGGCCCGAAAACGAATTGGCTTTGCTGTCAGCGCTTGCCATGTG
CACGATGAGTTCAGGACGGCAGCGGTGGAAGGCCCTTTTGTCACCTTGGACATGGA
AGACTGTGGCTACAACATTCCACAGACAGATGAGTCAACCCTCATGACCATAGCCTA
TGTCATGGCTGCCATCTGCGCCCTCTTCATGCTGCCACTCTGCCTCATGGTGTGTCA
GTGGCGCTGCCTCCGCTGCCTGCGCCAGCAGCATGATGACTTTGCTGATGACATCT
CCCTGCTGAAGTGAGGAGGCCCATGGGCAGAAGATAGAGATTCCCCTGGACCACAC
CTCCGTGGTTCACTTTGGTCACAAGTAGGAGACACAGATGGCACCTGTGGCCAGAG
CACCTCAGGACCCTCCCCACCCACCAAATGCCTCTGCCTTGATGGAGAAGGAAAAG
GCTGGCAAGGTGGGTTCCAGGGACTGTACCTGTAGGAAACAGAAAAGAGAAGAAAG
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GCTGCTTGAAACTTCAGCCCTGAACCTTTGTCCACCATTCCTTTAAATTCTCCAACCC
AAAGTATTCTTCTTTTCTTAGTTTCAGAAGTACTGGCATCACACGCAGGTTACCTTGG
CGTGTGTCCCTGTGGTACCCTGGCAGAGAAGAGACCAAGCTTGTTTCCCTGCTGGC
CAAAGTCAGTAGGAGAGGATGCACAGTTTGCTATTTGCTTTAGAGACAGGGACTGTA
TAAACAAGCCTAACATTGGTGCAAAGATTGCCTCTTGAATT

FIG. 1B

MAQALPWLLLWMGAGVLPAHGTQHGIRLPLRSGLGGAPLGLRLP

RETDEEPEEPGRRGSFVEMVDNLRGKSGQGYYVEMTVGSPPQT

LNILVDTGSSNFAVGAAPHPFLHRYYQRQLSSTYRDLRKGVYVPY 132

TQGKWEGELGTDLVSIPHGPNVTVRANIAAITESDKFFINGSNWE

GILGLAYAEIARPDDSLEPFFDSLVKQTHVPNLFSLQLCGAGFPLN

QSEVLASVGGSMIIGGIDHSLYTGSLWYTPIRREWYYEVIIVRVEIN

GQDLKMDCKEYNYDKSIVDSGTTNLRLPKKVFEAAVKSIKAASST

EKFPDGFWLGEQLVCWQAGTTPWNIFPVISLYLMGEVTNQSFRIT

ILPQQYLRPVEDVATSQDDCYKFAISQSSTGTVMGAVIMEGFYVV

FDRARKRIGFAVSACHVHDEFRTAAVEGPFVTLDMEDCGYNIPQ

TDESTLMTIAYVMAAICALFMLPLCLMVCQWRCLRCLRQQHDDF

ADDISLLK

FIG. 2A

ETDEEPEEPGRRGSFVEMVDNLRGKSGQGYYVEMTVGSPPQT
LNILVDTGSSNFAVGAAPHPFLHRYYQRQLSSTYRDLRKGVYVPY
TQGKWEGELGTDLVSIPHGPNVTVRANIAAITESDKFFINGSNWE
GILGLAYAEIARPDDSLEPFFDSLVKQTHVPNLFSLQLCGAGFPLN
QSEVLASVGGSMIIGGIDHSLYTGSLWYTPIRREWYYEVIIVRVEIN
GQDLKMDCKEYNYDKSIVDSGTTNLRLPKKVFEAAVKSIKAASST
EKFPDGFWLGEQLVCWQAGTTPWNIFPVISLYLMGEVTNQSFRIT
ILPQQYLRPVEDVATSQDDCYKFAISQSSTGTVMGAVIMEGFYVV
FDRARKRIGFAVSACHVHDEFRTAAVEGPFVTLDMEDCGYNIPQ
TDESTLMTIAYVMAAICALFMLPLCLMVCQWRCLRCLRQQHDDF
ADDISLLK

FIG. 2B

FIG. 3A

MAQALPWLLLWMGAGVLPAHGTQHGIRLPLRSGLGGAPLGLRLPRETDEEPE
EPGRRGSFVEMVDNLRGKSGQGYYVEMT
VGSPPQTLNILVDTGSSNFAVGAAPHPFLHRYYQRQLSSTYRDLRKGVYVPYT
QGKWEGELGTDLVSIPHGPNVTVRANI
AAITESDKFFINGSNWEGILGLAYAEIARPDDSLEPFFDSLVKQTHVPNLFSLQL
CGAGFPLNQSEVLASVGGSMIIGGI
DHSLYTGSLWYTPIRREWYYEVIIVRVEINGQDLKMDCKEYNYDKSIVDSGTTNL
RLPKKVFEAAVKSIKAASSTEKFPD
GFWLGEQLVCWQAGTTPWNIFPVISLYLMGEVTNQSFRITILPQQYLRPVEDVA
TSQDDCYKFAISQSSTGTVMGAVIME
GFYVVFDRARKRIGFAVSACHVHDEFRTAAVEGPFVTLDMEDCGYNIPQTDED
YKDDDDK

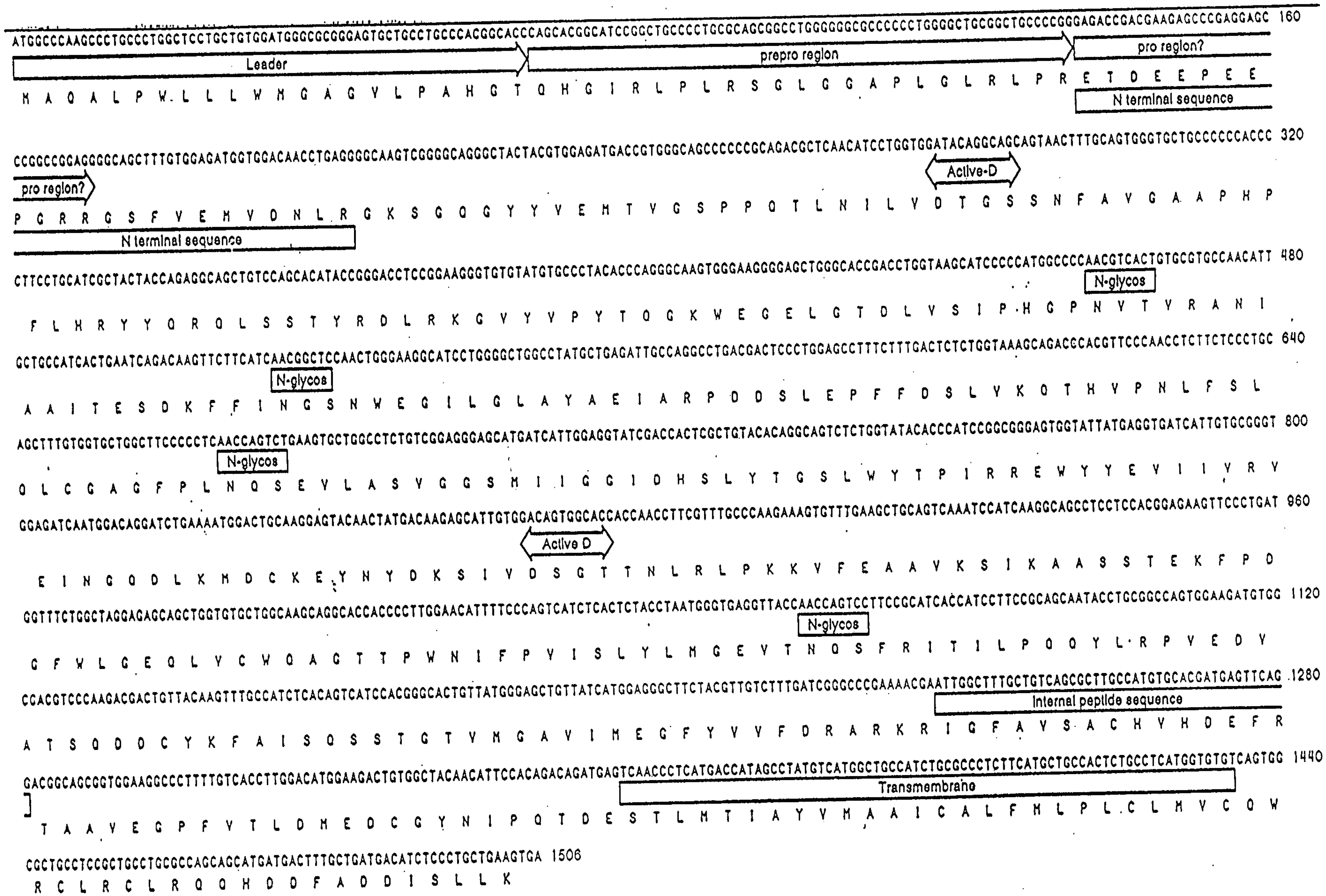
FIG. 3B

ETDEEPEEPGRRGSFVEMVDNLRGKSGQGYYVEMT
VGSPPQTLNILVDTGSSNFAVGAAPHPFLHRYYQRQLSSTYRDLRKGVYVPYT
QGKWEGELGTDLVSIPHGPNVTVRANI
AAITESDKFFINGSNWEGILGLAYAEIARPDDSLEPFFDSLVKQTHVPNLFSLQL
CGAGFPLNQSEVLASVGGSMIIGGI
DHSLYTGSLWYTPIRREWYYEVIIVRVEINGQDLKMDCKEYNYDKSIVDSGTTNL
RLPKKVFEAAVKSIKAASSTEKFPD
GFWLGEQLVCWQAGTTPWNIFPVISLYLMGEVTNQSFRITILPQQYLRPVEDVA
TSQDDCYKFAISQSSTGTVMGAVIME
GFYVVFDRARKRIGFAVSACHVHDEFRTAAVEGPFVTLDMEDCGYNIPQTDED
YKDDDDK
C

FIG. 4

P10 P1 P1' P4'

NH2-K-T-E-E-I-S-E-V-N-Sta-V-A-E-F-COOH



160
Leader
prepro region
pro region?
M A Q A L P W L L L W M G A G V L P A H G T Q H G I R L P L R S G L G G A P L G L R L P R E T D E E P E E
N terminal sequence
320
Active-D
pro region?
P G R R G S F V E M V D N L R G K S G Q G Y Y V E M T V G S P P Q T L N I L V D T G S S N F A V G A A P H P
N terminal sequence
480
N-glycos
F L H R Y Y Q R Q L S S T Y R D L R K G V Y V P Y T Q G K W E G E L G T D L V S I P H G P N V T V R A N I
640
N-glycos
A A I T E S D K F F I N G S N W E G I L G L A Y A E I A R P D D S L E P F F D S L V K Q T H V P N L F S L
800
N-glycos
Q L C G A G F P L N Q S E V L A S V G G S M I I G G I D H S L Y T G S L W Y T P I R R E W Y Y E V I I V R V
960
Active D
E I N G Q D L K M D C K E Y N Y D K S I V D S G T T N L R L P K K V F E A A V K S I K A A S S T E K F P D
1120
N-glycos
G F W L G E Q L V C W Q A G T T P W N I F P V I S L Y L M G E V T N Q S F R I T I L P Q Q Y L R P V E D V
1280
Internal peptide sequence
A T S Q D D C Y K F A I S Q S S T G T V M G A V I M E G F Y V V F D R A R K R I G F A V S A C H V H D E F R
1440
Transmembrane
T A A V E G P F V T L D M E D C G Y N I P Q T D E S T L M T I A Y V M A A I C A L F M L P L C L M V C Q W
1506
R C L R C L R Q Q H D D F A D D I S L L K

FIG. 5

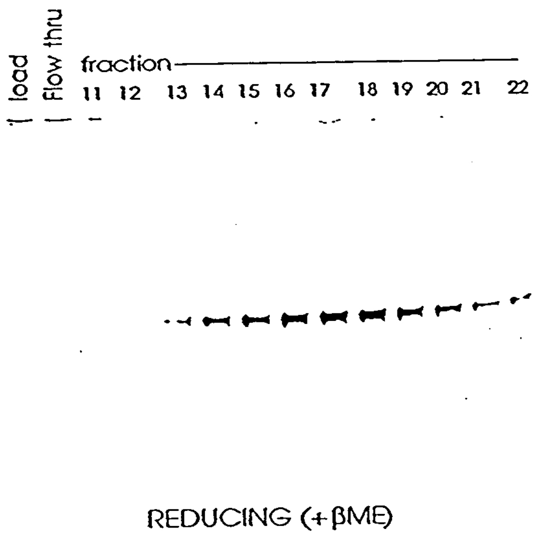

load
Flow thru
fraction
11 12 13 14 15 16 17 18 19 20 21 22
REDUCING (+βME)

FIG. 6A

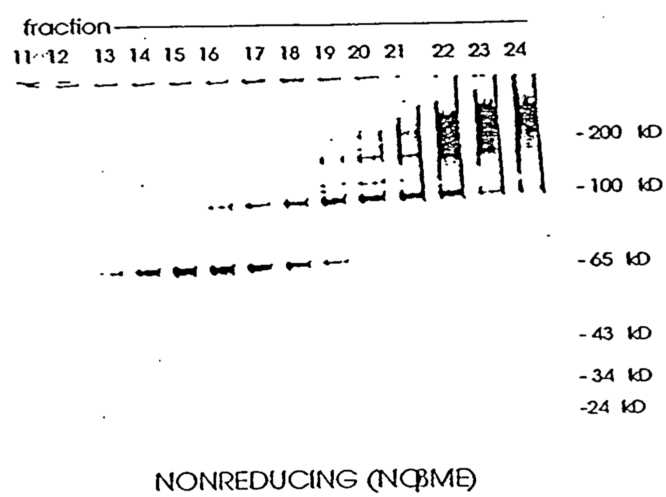

fraction
11 12 13 14 15 16 17 18 19 20 21 22 23 24
-200 kD
-100 kD
-65 kD
-43 kD
-34 kD
-24 kD
NONREDUCING (NOβME)

FIG. 6B

FIG. 7

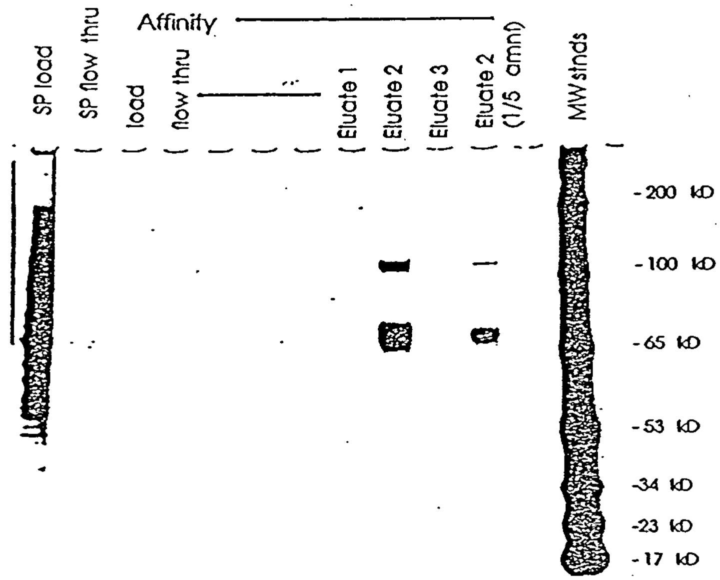
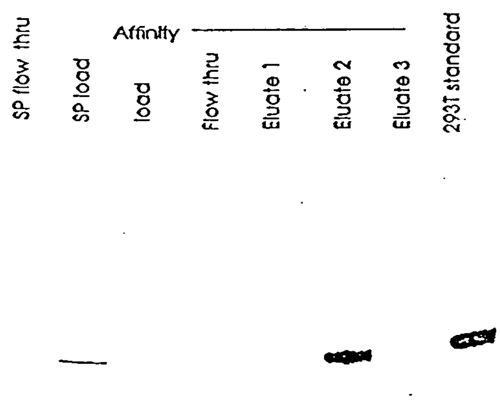
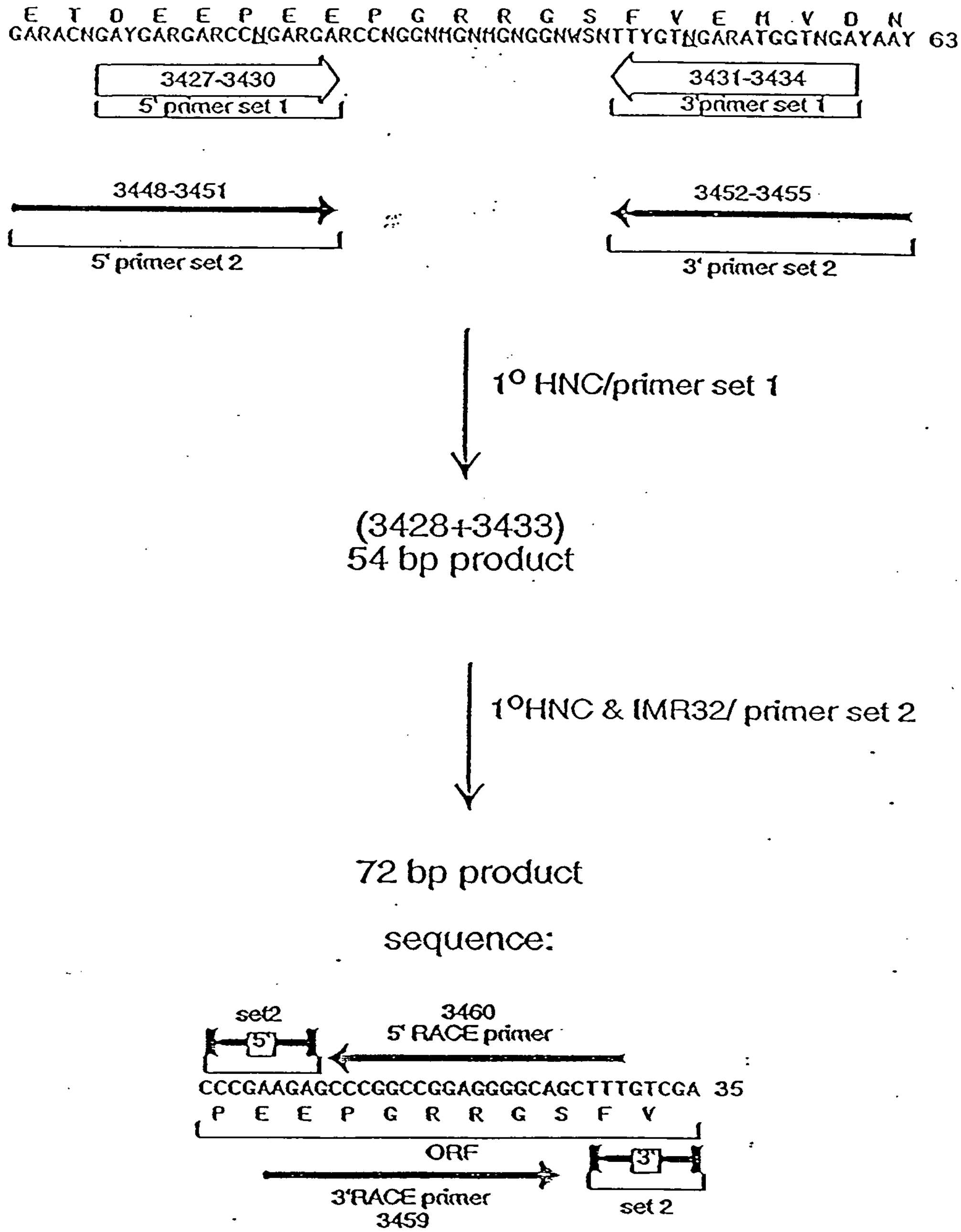

Affinity
SP load
SP flow thru
load
flow thru
Eluate 1
Eluate 2
Eluate 3
Eluate 2 (1/5 amnt)
MW stnds
-200 kD
-100 kD
-65 kD
-53 kD
-34 kD
-23 kD
-17 kD

FIG. 8


Affinity
SP flow thru
SP load
load
Flow thru
Eluate 1
Eluate 2
Eluate 3
293T standard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GARACNGAYGARGARCCNGARGARCCNGGNHGNHGNGGNWSNTTYGTNGARATGGTNGAYAAY 63
3427-3430
5' primer set 1
3431-3434
3' primer set 1
3448-3451
5' primer set 2
3452-3455
3' primer set 2
1° HNC/primer set 1
(3428+3433)
54 bp product
1°HNC & IMR32/ primer set 2
72 bp product
sequence:
set2
5'
3460
5' RACE primer
CCCGAAGAGCCCGGCCGGAGGGGCAGCTTTGTCGA 35
P E E P G R R G S F V
ORF
3'
3'RACE primer
3459
set 2

FIG. 9

FIG. 10

```
Human Imapain.seq              .........MAQALPWLLLWMGAGVLPAHGTQHGIRLPLRSGLGGAPLGLRLPRETDEEPEEPGRRGSFV  181
pBS/MuImPain E17 #11 cons      XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXSISLIEPGRRGSFV  103
pBS/MuDAPain E17#14 cons       .........................................................KLD.EPGRRGSFV   37
pBS/MU DAPain Brain #17 cons   XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXFV    4
pBS/MU DAPain Brain #15 cons   ....................................................................FV    4
pBS/MuImPain H#3 cons          .......................................................IDKLD.EPGRRGSFV    0

Human Imapain.seq              EMVDNLRGKSGQGYYVEMTVGSPPQTLNILVDTGSSNFAVGAAPHPFLHRYYQRQLSSTYRDLRKGVYVP  391
pBS/MuImPain E17 #11 cons      EMVNNLRGKSGQGYYVEMTVGSPPQTLNILVDTGSSNFAVGAAPHPFLHRYYQRQLSSTYRDLRKGVYVP  313
pBS/MuDAPain E17#14 cons       EMVDNLRGKSGQGYYVEMTVGSPPQTLNILVDTGSSNFAVGAAPHPFLHRYYQRQLSSTYRDLRKGVYVP  247
pBS/MU DAPain Brain #17 cons   EMVDNLRGKSGQGYYVEMTVGSPPQTLNILVDTGSSNFAVGAAPHPFLHRYYQRQLSSTYRDLRKGVYVP  214
pBS/MU DAPain Brain #15 cons   EMVDNLRGKSGQGYYVEMTVGSPPQTLNILVDTGSSNFAVGAAPHPFLHRYYQRQLSSTYRDLRKGVYVP  214
pBS/MuImPain H#3 cons          EMVDNLRGKSGQGYYVEMTVGSPPQTLNILVDTGSSNFAVGAAPHPFLHRYYQRQLSSTYRDLRKGVYVP  253

Human Imapain.seq              YTQGKWEGELGTDLVSIPHGPNVTVRANIAAITESDKFFINGSNWEGILGLAYAEIARPDDSLEPFFDSL  601
pBS/MuImPain E17 #11 cons      YTQGKWEGELGTDLVSIPHGPNVTVRANIAAITESDKFFINGSNWEGILGLAYAEIARPDDSLEPFFDSL  523
pBS/MuDAPain E17#14 cons       YTQGKWEGELGTDLVSIPHGPNVTVRANIAAITESDKFFINGSNWEGILGLAYAEIARPDDSLEPFFDSL  457
pBS/MU DAPain Brain #17 cons   YTQGKWEGELGTDLVSIPHGPNVTVRANIAAITESDKFFVNGSNWEGILGLAYAEIARPDDSLEPFFDSL  424
pBS/MU DAPain Brain #15 cons   YTQGKWEGELGTDLVSIPHGPNVTVRANIAAITESDKFFINGSNWEGILGLAYAEIARPDDSLEPFFDSL  424
pBS/MuImPain H#3 cons          YTQGKWEGELGTDLVSIPHGPNVTVRANIAAITESDKFFINGSNWEGILGLAYAEIARPDDSLEPFFDSL  463

Human Imapain.seq              VKQTHVPNLFSLQLCGAGFPLNQSEVLASVGGSMIIGGIDHSLYTGSLWYTPIRREWYYEVIIVRVEING  811
pBS/MuImPain E17 #11 cons      VKQTHIPNIFSLQLCGAGFPLNQTEALASVGGSMIIGGIDHSLYTGSLWYTPIRREWYYEVIIVRVEING  733
pBS/MuDAPain E17#14 cons       VKQTHIPNIFSLQLCGAGFPLNQTEALASVGGSMIIGGIDHSLYTGSLWYTPIRREWYYEVIIVRVEING  667
pBS/MU DAPain Brain #17 cons   VKQTHIPNIFSLQLCGAGFPLNQTEALASVGGSMIIGGIDHSLYTGSLWYTPIRREWYYEVIIVRVEING  634
pBS/MU DAPain Brain #15 cons   VKQTHIPNIFSLQLCGAGFPLNQTEALASVGGSMIIGGIDHSLYTGSLWYTPIRREWYYEVIIVRVEING  634
pBS/MuImPain H#3 cons          VKQTHIPNIFSLQLCGAGFPLNQTEALASVGGSMIIGGIDHSLYTGSLWYTPIRREWYYEVIIVRVEING  673

Human Imapain.seq              QDLKMDCKEYNYDKSIVDSGTTNLRLPKKVFEAAVKSIKAASSTEKFPDGFWLGEQLVCWQAGTTPWNIF  1021
pBS/MuImPain E17 #11 cons      QDLKMDCKEYNYDKSIVDSGTTNLRLPKKVFEAAVKSIKAASSTEKFPDGFWLGEQLVCWQAGTTPWNIF  943
pBS/MuDAPain E17#14 cons       QDLKMDCKEYNYDKSIVDSGTTNLRLPKKVFEAAVKSIKAASSTEKFPDGFWLGEQLVCWQAGTTPWNIF  877
pBS/MU DAPain Brain #17 cons   QDLKMDCKEYNYDKSIVDS                                                    694
pBS/MU DAPain Brain #15 cons   QDLKMDCKEYNYDKSIVDSGTTNLRLPKKVFEAAVKSIKAASSTEKFPDGFWLGEQLVCWQAGTTPWNIF  844
pBS/MuImPain H#3 cons          QDLKMDCKEYNYDKSIVDSGTTNLRLPKKVFEAAVKSIKAASSTEKFPDGFWLGEQLVCWQAGTTPWNIF  883

Human Imapain.seq              PVISLYLMGEVTNQSFRITILPQQYLRPVEDVATSQDDCYKFAISQSSTGTVMGAVIMEGFYVVFDRARK  1231
pBS/MuImPain E17 #11 cons      PVISLYLMGEVTNQSFRITILPQQYLRPVEDVATSQDDCYKFAVSQSSTGTVMGAVIMEGFYVVFDRARK  1153
pBS/MuDAPain E17#14 cons       PVISLYLMGEVTNQSFRITILPQQYLRPVEDVATSQDDCYKFAVSQSSTGTVMGAVIMEGFYVVFDRARK  1087
pBS/MU DAPain Brain #17 cons                                                                          694
pBS/MU DAPain Brain #15 cons   PVISLYLMGEVTNQSFRITILPQQYLRPVEDVATSQDDCYKFAVSQSSTGTVMGAVIMEGFYVVFDRARK  1054
pBS/MuImPain H#3 cons          PVISLYLMGEVTNQSFRITILPQQYLRPVEDVATSQDDCYKFAVSQSSTGTVMGAVIMEGFYVVFDRARK  1093

Human Imapain.seq              RIGFAVSACHVHDEFRTAAVEGPFVTLDMEDCGYNIPQTDESTLMTIAYVMAAICALFMLPLCLMVCQWR  1441
pBS/MuImPain E17 #11 cons      RIGFAVSACHVHDEFRTAAVEGPFVTADMEDCGYNNRIPAARGI                            1288
pBS/MuDAPain E17#14 cons       RIGFAVSACHVHDEFRTAAVEGPFVTADMEDCGYNNRIQ                                 1204
pBS/MU DAPain Brain #17 cons                                                                          694
pBS/MU DAPain Brain #15 cons   RIGFAVSACHVHDEFRTAAVEGPFVTAD                                            1138
pBS/MuImPain H#3 cons          RIGFAVSACHVHDEFRTAAVEGPFVTADMED.GYNNRIPAARGIH.F.SGRHRGGAPIRPIVSRIN      1291

Human Imapain.seq              CLRCLRQQHDDFADDISLLK.                                                   1504
pBS/MuImPain E17 #11 cons                                                                              1288
pBS/MuDAPain E17#14 cons                                                                               1204
pBS/MU DAPain Brain #17 cons                                                                           694
pBS/MU DAPain Brain #15 cons                                                                           1138
pBS/MuImPain H#3 cons                                                                                  1291
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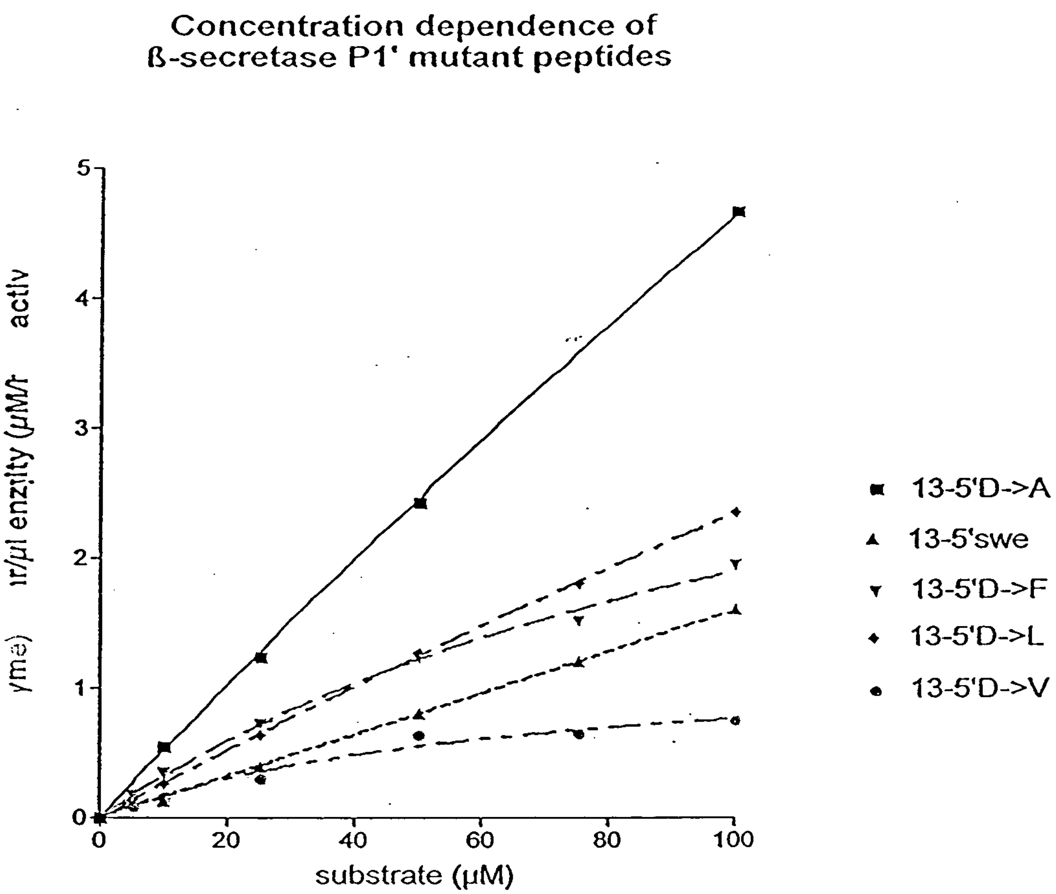
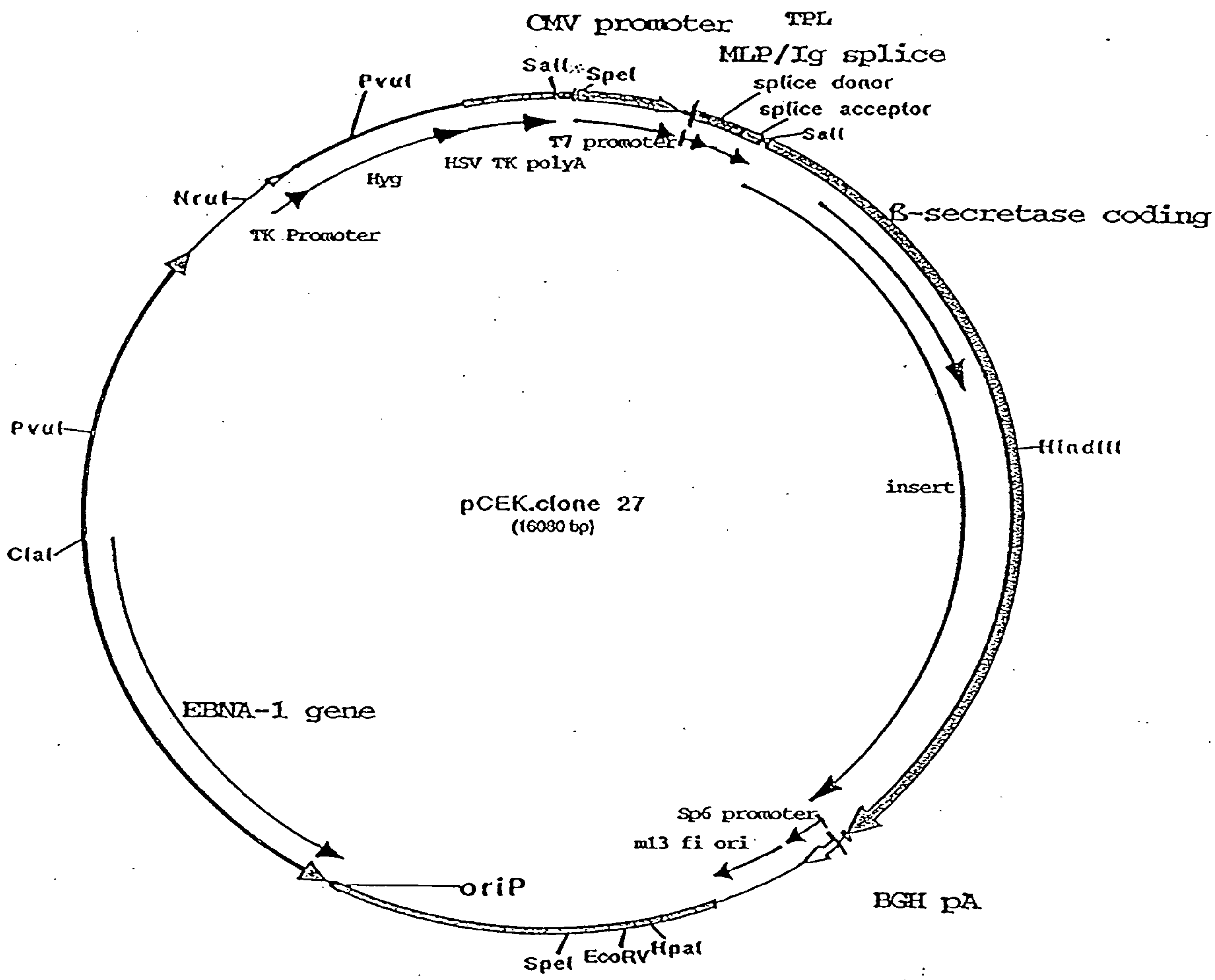

Concentration dependence of
ß-secretase P1' mutant peptides
ymə) ır/µl enzıty (µM/r activ
0
1
2
3
4
5
0
20
40
60
80
100
substrate (µM)
13-5'D->A
13-5'swe
13-5'D->F
13-5'D->L
13-5'D->V

FIG. 11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TPL
MLP/Ig splice
splice donor
splice acceptor
SalI
SpeI
SalI
PvuI
T7 promoter
HSV TK polyA
Hyg
NruI
TK Promoter
ß-secretase coding
HindIII
insert
PvuI
ClaI
pCEK.clone 27
(16080 bp)
EBNA-1 gene
Sp6 promoter
m13 fi ori
oriP
BGH pA
SpeI
EcoRV
HpaI

FIG. 12

FIG. 13A

```
1    TTCTCATGTTTGACAGCTTATCATCGCAGATCCGGGCAACGTTGTTGCATTGCTGCAGGCGCAGAACTGGTAGGTATGGAAGATCCGATGTACGGGCCAGATATAC
                        SpeI
107  GCGTTGACATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGC
213  CCGCCTGGCTGACCGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCATTGACGTCAATGGGTGGACT
319  ATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCA
425  GTACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAG
531  CGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTCGTAACAACTCCG
637  CCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGGTCTATATAAGCAGAGCTCTCTGGCTAACTAGAGAACCCACTGCTTACTGGCTTATCGAAATTAA
743  TACGACTCACTATAGGGAGACCCAAGCTCTGTTGGGCTCGCGGTTGAGGACAAACTCTTCGCGGTCTTTCCAGTACTCTTGGATCGGAAACCCGTCGGCCTCCGAA
                                                                                      splice donor
849  CGGTACTCCGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGGATCGGAAAACCTCTCGACTGTTGGGGTGAGTACTCCCTCTCAAAAGCGGGCATGACTTCTG
955  CGCTAAGATTGTCAGTTTCCAAAAACGAGGAGGATTTGATATTCACCTGGCCCGCGGTGATGCCTTTGAGGGTGGCCGCGTCCATCTGGTCAGAAAAGACAATCTT
                                                                                                  splice acceptor
1061 TTTGTTGTCAAGCTTGAGGTGTGGCAGGCTTGAGATCTGGCCATACACTTGAGTGACAATGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGGTCC
           SalI
1167 AACTGCAGGTCGACTCTAGACCCGGGGAATTCTGCAGATATCCATCACACTGGCCGCACTCGTCCCCAGCCCGCCCGGGAGCTGCGAGCCGCGAGCTGGATTATGG
1273 TGGCCTGAGCAGCCAACGCAGCCGCAGGAGCCCGGAGCCCTTGCCCCTGCCCGCGCCGCCGCCCGCCGGGGGGACCAGGGAAGCCGCCACCGGCCCGCCATGCCCG
1379 CCCCTCCCAGCCCCGCCGGGAGCCCGCGCCCGCTGCCCAGGCTGGCCGCCGCCGTGCCGATGTAGCGGGCTCCGGATCCCAGCCTCTCCCCTGCTCCCGTGCTCTG
1485 CGGATCTCCCCTGACCGCTCTCCACAGCCCGGACCCGGGGGCTGGCCCAGGGCCCTGCAGGCCCTGGCGTCCTGATGCCCCCAAGCTCCCTCTCCTGAGAAGCCAC
1591 CAGCACCACCCAGACTTGGGGGCAGGCGCCAGGGACGGACGTGGGCCAGTGCGAGCCCAGAGGGCCCGAAGGCCGGGGCCCACC ATG GCC CAA GCC CTG
                                                                                      1 Met Ala Gln Ala Leu

1690 CCC TGG CTC CTG CTG TGG ATG GGC GCG GGA GTG CTG CCT GCC CAC GGC ACC CAG CAC GGC ATC CGG CTG CCC CTG CGC
   6 Pro Trp Leu Leu Leu Trp Met Gly Ala Gly Val Leu Pro Ala His Gly Thr Gln His Gly Ile Arg Leu Pro Leu Arg
1768 AGC GGC CTG GGG GGC GCC CCC CTG GGG CTG CGG CTG CCC CGG GAG ACC GAC GAA GAG CCC GAG GAG CCC GGC CGG AGG
  32 Ser Gly Leu Gly Gly Ala Pro Leu Gly Leu Arg Leu Pro Arg Glu Thr Asp Glu Glu Pro Glu Glu Pro Gly Arg Arg
1846 GGC AGC TTT GTG GAG ATG GTG GAC AAC CTG AGG GGC AAG TCG GGG CAG GGC TAC TAC GTG GAG ATG ACC GTG GGC AGC
  58 Gly Ser Phe Val Glu Met Val Asp Asn Leu Arg Gly Lys Ser Gly Gln Gly Tyr Tyr Val Glu Met Thr Val Gly Ser
1924 CCC CCG CAG ACG CTC AAC ATC CTG GTG GAT ACA GGC AGC AGT AAC TTT GCA GTG GGT GCT GCC CCC CAC CCC TTC CTG
  84 Pro Pro Gln Thr Leu Asn Ile Leu Val Asp Thr Gly Ser Ser Asn Phe Ala Val Gly Ala Ala Pro His Pro Phe Leu
2002 CAT CGC TAC TAC CAG AGG CAG CTG TCC AGC ACA TAC CGG GAC CTC CGG AAG GGT GTG TAT GTG CCC TAC ACC CAG GGC
 110 His Arg Tyr Tyr Gln Arg Gln Leu Ser Ser Thr Tyr Arg Asp Leu Arg Lys Gly Val Tyr Val Pro Tyr Thr Gln Gly
2080 AAG TGG GAA GGG GAG CTG GGC ACC GAC CTG GTA AGC ATC CCC CAT GGC CCC AAC GTC ACT GTG CGT GCC AAC ATT GCT
 136 Lys Trp Glu Gly Glu Leu Gly Thr Asp Leu Val Ser Ile Pro His Gly Pro Asn Val Thr Val Arg Ala Asn Ile Ala
2158 GCC ATC ACT GAA TCA GAC AAG TTC TTC ATC AAC GGC TCC AAC TGG GAA GGC ATC CTG GGG CTG GCC TAT GCT GAG ATT
 162 Ala Ile Thr Glu Ser Asp Lys Phe Phe Ile Asn Gly Ser Asn Trp Glu Gly Ile Leu Gly Leu Ala Tyr Ala Glu Ile
2236 GCC AGG CCT GAC GAC TCC CTG GAG CCT TTC TTT GAC TCT CTG GTA AAG CAG ACC CAC GTT CCC AAC CTC TTC TCC CTG
 188 Ala Arg Pro Asp Asp Ser Leu Glu Pro Phe Phe Asp Ser Leu Val Lys Gln Thr His Val Pro Asn Leu Phe Ser Leu
2314 CAG CTT TGT GGT GCT GGC TTC CCC CTC AAC CAG TCT GAA GTG CTG GCC TCT GTC GGA GGG AGC ATG ATC ATT GGA GGT
 214 Gln Leu Cys Gly Ala Gly Phe Pro Leu Asn Gln Ser Glu Val Leu Ala Ser Val Gly Gly Ser Met Ile Ile Gly Gly
2392 ATC GAC CAC TCG CTG TAC ACA GGC AGT CTC TGG TAT ACA CCC ATC CGG CGG GAG TGG TAT TAT GAG GTC ATC ATT GTG
 240 Ile Asp His Ser Leu Tyr Thr Gly Ser Leu Trp Tyr Thr Pro Ile Arg Arg Glu Trp Tyr Tyr Glu Val Ile Ile Val
2470 CGG GTG GAG ATC AAT GGA CAG GAT CTG AAA ATG GAC TGC AAG GAG TAC AAC TAT GAC AAG AGC ATT GTG GAC AGT GGC
 266 Arg Val Glu Ile Asn Gly Gln Asp Leu Lys Met Asp Cys Lys Glu Tyr Asn Tyr Asp Lys Ser Ile Val Asp Ser Gly
2548 ACC ACC AAC CTT CGT TTG CCC AAG AAA GTG TTT GAA GCT GCA GTC AAA TCC ATC AAG GCA GCC TCC TCC ACG GAG AAG
 292 Thr Thr Asn Leu Arg Leu Pro Lys Lys Val Phe Glu Ala Ala Val Lys Ser Ile Lys Ala Ala Ser Ser Thr Glu Lys
2626 TTC CCT GAT GGT TTC TGG CTA GGA GAG CAG CTG GTG TGC TGG CAA GCA GGC ACC ACC CCT TGG AAC ATT TTC CCA GTC
 318 Phe Pro Asp Gly Phe Trp Leu Gly Glu Gln Leu Val Cys Trp Gln Ala Gly Thr Thr Pro Trp Asn Ile Phe Pro Val
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FIG. 13B

2704 ATC TCA CTC TAC CTA ATG GGT GAG GTT ACC AAC CAG TCC TTC CGC ATC ACC ATC CTT CCG CAG CAA TAC CTG CGG CCA
344▸ Ile Ser Leu Tyr Leu Met Gly Glu Val Thr Asn Gln Ser Phe Arg Ile Thr Ile Leu Pro Gln Gln Tyr Leu Arg Pro

2782 GTG GAA GAT GTG GCC ACG TCC CAA GAC GAC TGT TAC AAG TTT GCC ATC TCA CAG TCA TCC ACG GGC ACT GTT ATG GGA
370▸ Val Glu Asp Val Ala Thr Ser Gln Asp Asp Cys Tyr Lys Phe Ala Ile Ser Gln Ser Ser Thr Gly Thr Val Met Gly

2860 GCT GTT ATC ATG GAG GGC TTC TAC GTT GTC TTT GAT CGG GCC CGA AAA CGA ATT GGC TTT GCT GTC AGC GCT TGC CAT
396▸ Ala Val Ile Met Glu Gly Phe Tyr Val Val Phe Asp Arg Ala Arg Lys Arg Ile Gly Phe Ala Val Ser Ala Cys His

2938 GTG CAC GAT GAG TTC AGG ACG GCA GCG GTG GAA GGC CCT TTT GTC ACC TTG GAC ATG GAA GAC TGT GGC TAC AAC ATT
422▸ Val His Asp Glu Phe Arg Thr Ala Ala Val Glu Gly Pro Phe Val Thr Leu Asp Met Glu Asp Cys Gly Tyr Asn Ile

3016 CCA CAG ACA GAT GAG TCA ACC CTC ATG ACC ATA GCC TAT GTC ATG GCT GCC ATC TGC GCC CTC TTC ATG CTG CCA CTC
448▸ Pro Gln Thr Asp Glu Ser Thr Leu Met Thr Ile Ala Tyr Val Met Ala Ala Ile Cys Ala Leu Phe Met Leu Pro Leu

3094 TGC CTC ATG GTG TGT CAG TGG CGC TGC CTC CGC TGC CTG CGC CAG CAG CAT GAT GAC TTT GCT GAT GAC ATC TCC CTG
474▸ Cys Leu Met Val Cys Gln Trp Arg Cys Leu Arg Cys Leu Arg Gln Gln His Asp Asp Phe Ala Asp Asp Ile Ser Leu

3172 CTG AAG TGA GGAGGCCCATGGGCAGAAGATAGAGATTCCCCTGGACCACACCTCCGTGGTTCACTTTGGTCACAAGTAGGAGACACAGATGGCACCTGTGGCC
500▸ Leu Lys •••

3275 AGAGCACCTCAGGACCCTCCCCACCCACCAAATGCCTCTGCCTTGATGGAGAAGGAAAAGGCTGGCAAGGTGGGTTCCAGGGACTGTACCTGTAGGAAACAGAAAA

3381 GAGAAGAAAGAAGCACTCTGCTGGCGGGAATACTCTTGGTCACCTCAAATTTAAGTCGGGAAATTCTGCTGCTTGAAACTTCAGCCCTGAACCTTTGTCCACCATT

3487 CCTTTAAATTCTCCAACCCAAAGTATTCTTCTTTTCTTAGTTTCAGAAGTACTGGCATCACACGCAGGTTACCTTGGCGTGTGTCCCTGTGGTACCCTGGCAGAGA

HindIII
3593 AGAGACCAAGCTTGTTTCCCTGCTGGCCAAAGTCAGTAGGAGAGGATGCACAGTTTGCTATTTGCTTTAGAGACAGGGACTGTATAAACAAGCCTAACATTGGTGC

3699 AAAGATTGCCTCTTGAATTAAAAAAAAAAACTAGATTGACTATTTATACAAATGGGGGCGGCTGGAAAGAGGAGAAGGAGAGGGAGTACAAAGACAGGGAATAGTG

3805 GGATCAAAGCTAGGAAAGGCAGAAACACAACCACTCACCAGTCCTAGTTTTAGACCTCATCTCCAAGATAGCATCCCATCTCAGAAGATGGGTGTTGTTTTCAATG

3911 TTTTCTTTTCTGTGGTTGCAGCCTGACCAAAAGTGAGATGGGAAGGGCTTATCTAGCCAAAGAGCTCTTTTTTAGCTCTCTTAAATGAAGTGCCCACTAAGAAGTT

4017 CCACTTAACACATGAATTTCTGCCATATTAATTTCATTGTCTCTATCTGAACCACCCTTTATTCTACATATGATAGGCAGCACTGAAATATCCTAACCCCCTAAGC

4123 TCCAGGTGCCCTGTGGGAGAGCAACTGGACTATAGCAGGGCTGGGCTCTGTCTTCCTGGTCATAGGCTCACTCTTTCCCCCAAATCTTCCTCTGGAGCTTTGCAGC

4229 CAAGGTGCTAAAAGGAATAGGTAGGAGACCTCTTCTATCTAATCCTTAAAAGCATAATGTTGAACATTCATTCAACAGCTGATGCCCTATAACCCCTGCCTGGATT

4335 TCTTCCTATTAGGCTATAAGAAGTAGCAAGATCTTTACATAATTCAGAGTGGTTTCATTGCCTTCCTACCCTCTCTAATGGCCCCTCCATTTATTTGACTAAAGCA

4441 TCACACAGTGGCACTAGCATTATACCAAGAGTATGAGAAATACAGTGCTTTATGGCTCTAACATTACTGCCTTCAGTATCAAGGCTGCCTGGAGAAAGGATGGCAG

4547 CCTCAGGGCTTCCTTATGTCCTCCACCACAAGAGCTCCTTGATGAAGGTCATCTTTTTCCCCTATCCTGTTCTTCCCCTCCCCGCTCCTAATGGTACGTGGGTACC

4653 CAGGCTGGTTCTTGGGCTAGGTAGTGGGGACCAAGTTCATTACCTCCCTATCAGTTCTAGCATAGTAAACTACGGTACCAGTGTTAGTGGGAAGAGCTGGGTTTTC

4759 CTAGTATACCCACTGCATCCTACTCCTACCTGGTCAACCCGCTGCTTCCAGGTATGGGACCTGCTAAGTGTGGAATTACCTGATAAGGGAGAGGGAAATACAAGGA

4865 GGGCCTCTGGTGTTCCTGGCCTCAGCCAGCTGCCCACAAGCCATAAACCAATAAAACAAGAATACTGAGTCAGTTTTTTATCTGGGTTCTCTTCATTCCCACTGCA

4971 CTTGGTGCTGCTTTGGCTGACTGGGAACACCCCATAACTACAGAGTCTGACAGGAAGACTGGAGACTGTCCACTTCTAGCTCGGAACTTACTGTGTAAATAAACTT

5077 TCAGAACTGCTACCATGAAGTGAAAATGCCACATTTTGCTTTATAATTTCTACCCATGTTGGGAAAAACTGGCTTTTTCCCAGCCCTTTCCAGGGCATAAAACTCA

5183 ACCCCTTCGATAGCAAGTCCCATCAGCCTATTATTTTTTTAAAGAAAACTTGCACTTGTTTTTCTTTTTACAGTTACTTCCTTCCTGCCCCAAAATTATAAACTCT

FIG. 13C

5289 AAGTGTAAAAAAAAGTCTTAACAACAGCTTCTTGCTTGTAAAAATATGTATTATACATCTGTATTTTTAAATTCTGCTCCTGAAAAATGACTGTCCCATTCTCCAC

5395 TCACTGCATTTGGGGCCTTTCCCATTGGTCTGCATGTCTTTTATCATTGCAGGCCAGTGGACAGAGGGAGAAGGGAGAACAGGGGTCGCCAACACTTGTGTTGCTT

5501 TCTGACTGATCCTGAACAAGAAAGAGTAACACTGAGGCGCTCGCTCCCATGCACAACTCTCCAAAACACTTATCCTCCTGCAAGAGTGGGCTTTCCGGGTCTTTAC

5607 TGGGAAGCAGTTAAGCCCCCTCCTCACCCCTTCCTTTTTTCTTTCTTTACTCCTTTGGCTTCAAAGGATTTTGGAAAAGAAACAATATGCTTTACACTCATTTTCA

5713 ATTTCTAAATTTGCAGGGGATACTGAAAAATACGGCAGGTGGCCTAAGGCTGCTGTAAAGTTGAGGGGAGAGGAAATCTTAAGATTACAAGATAAAAAACGAATCC

5819 CCTAAACAAAAAGAACAATAGAACTGGTCTTCCATTTTGCCACCTTTCCTGTTCATGACAGCTACTAACCTGGAGACAGTAACATTTCATTAACCAAAGAAAGTGG

5925 GTCACCTGACCTCTGAAGAGCTGAGTACTCAGGCCACTCCAATCACCCTACAAGATGCCAAGGAGGTCCCAGGAAGTCCAGCTCCTTAAACTGACGCTAGTCAATA

6031 AACCTGGGCAAGTGAGGCAAGAGAAATGAGGAAGAATCCATCTGTGAGGTGACAGGCACGGATGAAAGACAAAGACGGAAAAGAGTATCAAAGGCAGAAAGGAGAT

6137 CATTTAGTTGGGTCTGAAAGGAAAAGTNTTTGCTATCCGACATGTACTGCTAGTWCCTGTAAGCATTTTAGGTCCCAGAATGGAAAAAAAAATCAAGCTATNGGTT

6243 ATATAATAATGNNNNNNNNNNNNNNNNNNNNNNnTCGAGCATGCATCTAGAGGGCCCTATTCTATAGTGTCACCTAAATGCTAGAGCTCGCTGATCAGCCTCGACTGTGC

6349 CTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATC

6455 GCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGC

6561 TCTATGGCTTCTGAGGCGGAAAGAACCAGCTGGGGCTCTAGGGGGTATCCCCACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCG

6667 TGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGCAT

6773 CCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGC

6879 CCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGTTCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGG

6985 GGATTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACGCGAATTCTAGAGCCCCGCCGCCGGACGAACTAAACCTGACTACGGCATCTCTGCC

7091 CCTTCTTCGCGGGGCAGTGCATGTAATCCCTTCAGTTGGTTGGTACAACTTGCCAACTGGGCCCTGTTCCACATGTGACACGGGGGGGGACCAAACACAAAGGGGT
7197 TCTCTGACTGTAGTTGACATCCTTATAAATGGATGTGCACATTTGCCAACACTGAGTGGCTTTCATCCTGGAGCAGACTTTGCAGTCTGTGGACTGCAACACAACA
7303 TTGCCTTTATGTGTAACTCTTGGCTGAAGCTCTTACACCAATGCTGGGGGACATGTACCTCCCAGGGGCCCAGGAAGACTACGGGAGGCTACACCAACGTCAATCA

HpaI

7409 GAGGGGCCTGTGTAGCTACCGATAAGCGGACCCTCAAGAGGGCATTAGCAATAGTGTTTATAAGGCCCCCTTGTTAACCCTAAACGGGTAGCATATGCTTCCCGGG
7515 TAGTAGTATATACTATCCAGACTAACCCTAATTCAATAGCATATGTTACCCAACGGGAAGCATATGCTATCGAATTAGGGTTAGTAAAAGGGTCCTAAGGAACAGC

EcoRV

7621 GATATCTCCCACCCCATGAGCTGTCACGGTTTTATTTACATGGGGTCAGGATTCCACGAGGGTAGTGAACCATTTTAGTCACAAGGGCAGTGGCTGAAGATCAAGG
7727 AGCGGGCAGTGAACTCTCCTGAATCTTCGCCTGCTTCTTCATTCTCCTTCGTTTAGCTAATAGAATAACTGCTGAGTTGTGAACAGTAAGGTGTATGTGAGGTGCT
7833 CGAAAACAAGGTTTCAGGTGACGCCCCCAGAATAAAATTTGGACGGGGGGTTCAGTGGTGGCATTGTGCTATGACACCAATATAACCCTCACAAACCCCTTGGGCA

SpeI

7939 ATAAATACTAGTGTAGGAATGAAACATTCTGAATATCTTTAACAATAGAAATCCATGGGGTGGGGACAAGCCGTAAAGACTGGATGTCCATCTCACACGAATTTAT
8045 GGCTATGGGCAACACATAATCCTAGTGCAATATGATACTGGGGTTATTAAGATGTGTCCCAGGCAGGGACCAAGACAGGTGAACCATGTTGTTACACTCTATTTGT
8151 AACAAGGGGAAAGAGAGTGGACGCCGACAGCAGCGGACTCCACTGGTTGTCTCTAACACCCCCGAAAATTAAACGGGGCTCCACGCCAATGGGGCCCATAAACAAA
8257 GACAAGTGGCCACTCTTTTTTTTGAAATTGTGGAGTGGGGGCACGCGTCAGCCCCCACACGCCGCCCTGCGGTTTTGGACTGTAAAATAAGGGTGTAATAACTTGG
8363 CTGATTGTAACCCCGCTAACCACTGCGGTCAAACCACTTGCCCACAAAACCACTAATGGCACCCCGGGGAATACCTGCATAAGTAGGTGGGCGGGCCAAGATAGGG
8469 GCGCGATTGCTGCGATCTGGAGGACAAATTACACACACTTGCGCCTGAGCGCCAAGCACAGGGTTGTTGGTCCTCATATTCACGAGGTCGCTGAGAGCACGGTGGG
8575 CTAATGTTGCCATGGGTAGCATATACTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGC
8681 ATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATC
8787 TATATCTGGGTAGTATATGCTATCCTAATCTGTATCCGGGTAGCATATGCTATCCTAATAGAGATTAGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCAT
8893 ATACTACCCAAATATCTGGATAGCATATGCTATCCTAATCTATATCTGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGCATAGGCTATCCTAATCTATATC
8999 TGGGTAGCATATGCTATCCTAATCTATATCTGGGTAGTATATGCTATCCTAATTTATATCTGGGTAGCATAGGCTATCCTAATCTATATCTGGGTAGCATATGCTA
9105 TCCTAATCTATATCTGGGTAGTATATGCTATCCTAATCTGTATCCGGGTAGCATATGCTATCCTCATGCATATACAGTCAGCATATGATACCCAGTAGTAGAGTGG

oriP

9211 GAGTGCTATCCTTTGCATATGCCGCCACCTCCCAAGGGGGCGTGAATTTTCGCTGCTTGTCCTTTTCCTGCATGCTGGTTGCTCCCATTCTTAGGTGAATTTAAGG

9317 AGGCCAGGCTAAAGCCGTCGCATGTCTGATTGCTCACCAGGTAAATGTCGCTAATGTTTTCCAACGCGAGAAGGTGTTGAGCGCGGAGCTGAGTGACGTGACAACA

9423 TGGGTATGCCCAATTGCCCCATGTTGGGAGGACGAAAATGGTGACAAGACAGATGGCCAGAAATACACCAACAGCACGCATGATGTCTACTGGGGATTTATTCTTT

9529 AGTGCGGGGGAATACACGGCTTTTAATACGATTGAGGGCGTCTCCTAACAAGTTACATCACTCCTGCCCTTCCTCACCCTCATCTCCATCACCTCCTTCATCTCCG

9635 TCATCTCCGTCATCACCCTCCGCGGCAGCCCCTTCCACCATAGGTGGAAACCAGGGAGGCAAATCTACTCCATCGTCAAAGCTGCACACAGTCACCCTGATATTGC

9741 AGGTAGGAGCGGGCTTTGTCATAACAAGGTCCTTAATCGCATCCTTCAAAACCTCAGCAAATATATGAGTTTGTAAAAAGACCATGAAATAACAGACAATGGACTC

FIG. 13D

9847 CCTTAGCGGGCCAGGTTGTGGGCCGGGTCCAGGGGCCATTCCAAAGGGGAGACGACTCAATGGTGTAAGACGACATTGTGGAATAGCAAGGGCAGTTCCTCGCCTT

9953 AGGTTGTAAAGGGAGGTCTTACTACCTCCATATACGAACACACCGGCGACCCAAGTTCCTTCGTCGGTAGTCCTTTCTACGTGACTCCTAGCCAGGAGAGCTCTTA

10059 AACCTTCTGCAATGTTCTCAAATTTCGGGTTGGAACCTCCTTGACCACGATGCTTTCCAAACCACCCTCCTTTTTTGCGCCTGCCTCCATCACCCTGACCCCGGGG

10165 TCCAGTGCTTGGGCCTTCTCCTGGGTCATCTGCGGGGCCCTGCTCTATCGCTCCCGGGGGCACGTCAGGCTCACCATCTGGGCCACCTTCTTGGTGGTATTCAAAA

10271 TAATCGGCTTCCCCTACAGGGTGGAAAAATGGCCTTCTACCTGGAGGGGGCCTGCGCGGTCGAGACCCGGATGATGATGACTGACTACTGGGACTCCTGGGCCTCT

10377 TTTCTCCACGTCCACGACCTCTCCCCCTGGCTCTTTCACGACTTCCCCCCCTGGCTCTTTCACGTCCTCTACCCCGGCGGCCTCCACTACCTCCTCGACCCCGGCC

10483 TCCACTACCTCCTCGACCCCGGCCTCCACTGCCTCCTCGACCCCGGCTCCACCTCCTGCTCCTGCCCCTCCTGCTCCTGCCCCTCCTCCTGCTCCTGCCCCTCCT

10589 GCCCCTCCTGCTCCTGCCCCTCCTGCCCCTCCTGCTCCTGCCCCTCCTGCCCCTCCTGCTCCTGCCCCTCCTGCCCCTCCTCCTGCTCCTGCCCCTCCTGCCCCTC

10695 CTCCTGCTCCTGCCCCTCCTGCCCCTCCTGCTCCTGCCCCTCCTGCCCCTCCTGCTCCTGCCCCTCCTGCCCCTCCTGCTCCTGCCCCTCCTGCTCCTGCCCCTCC

10801 TGCTCCTGCCCCTCCTGCTCCTGCCCCTCCTGCCCCTCCTGCCCCTCCTCCTGCTCCTGCCCCTCCTGCTCCTGCCCCTCCTGCCCCTCCTGCCCCTCCTGCTCCT

10907 GCCCCTCCTCCTGCTCCTGCCCCTCCTGCCCCTCCTGCCCCTCCTCCTGCTCCTGCCCCTCCTGCCCCTCCTCCTGCTCCTGCCCCTCCTCCTGCTCCTGCCCCTC

11013 CTGCCCCTCCTGCCCCTCCTCCTGCTCCTGCCCCTCCTGCCCCTCCTCCTGCTCCTGCCCCTCCTCCTGCTCCTGCCCCTCCTGCCCCTCCTGCCCCTCCTCCTGC

11119 TCCTGCCCCTCCTCCTGCTCCTGCCCCTCCTGCCCCTCCTGCCCCTCCTGCCCCTCCTCCTGCTCCTGCCCCTCCTCCTGCTCCTGCCCCTCCTGCTCCTGCCCCT

11225 CCCGCTCCTGCTCCTGCTCCTGTTCCACCGTGGGTCCCTTTGCAGCCAATGCAACTTGGACGTTTTTGGGGTCTCCGGACACCATCTCTATGTCTTGGCCCTGATC

11331 CTGAGCCGCCCGGGGCTCCTGGTCTTCCGCCTCCTCGTCCTCGTCCTCTTCCCCGTCCTCGTCCATGGTTATCACCCCCTCTTCTTTGAGGTCCACTGCCGCCGGA

11437 GCCTTCTGGTCCAGATGTGTCTCCCTTCTCTCCTAGGCCATTTCCAGGTCCTGTACCTGGCCCCTCGTCAGACATGATTCACACTAAAAGAGATCAATAGACATCT

11543 TTATTAGACGACGCTCAGTGAATACAGGGAGTGCAGACTCCTGCCCCCTCCAACAGCCCCCCCACCCTCATCCCCTTCATGGTCGCTGTCAGACAGATCCAGGTCT

11649 GAAAATTCCCCATCCTCCGAACCATCCTCGTCCTCATCACCAATTACTCGCAGCCCGGAAAACTCCCGCTGAACATCCTCAAGATTTGCGTCCTGAGCCTCAAGCC

11755 AGGCCTCAAATTCCTCGTCCCCCTTTTTGCTGGACGGTAGGGATGGGGATTCTCGGGACCCCTCCTCTTCCTCTTCAAGGTCACCAGACAGAGATGCTACTGGGGC

ClaI

11861 AACGGAAGAAAAGCTGGGTGCGGCCTGTGAGGATCAGCTTATCGATGATAAGCTGTCAAACATGAGAATTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTT

11967 TATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAA
12073 TATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTT
12179 GCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCA
12285 ACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGG
12391 GCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTA

PvuI

12497 TGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATC
12603 ATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACT
12709 ATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGC
12815 TGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGA
12921 CGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACT
13027 TTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGA
13133 GCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTT
13239 GTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCA
13345 CTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGA
13451 CGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTG
13557 AGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAA
13663 CGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAAC
13769 GCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCGCGTGCGGCTGCTGGAGATGGCGGACGCGATGGATATGTTCTGCCAAGGGTTGGTTTGCGCATTCACAGT
13875 TCTCCGCAAGAATTGATTGGCTCCAATTCTTGGAGTGGTGAATCCGTTAGCGAGGTGCCGCCGGCTTCCATTCAGGTCGAGGTGGCCCGGCTCCATGCACCGCGAC
13981 GCAACGCGGGGAGGCAGACAAGGTATAGGGCGGCGCCTACAATCCATGCCAACCCGTTCCATGTGCTCGCCGAGGCGGCATAAATCGCCGTGACGATCAGCGGTCC
14087 AGTGATCGAAGTTAGGCTGGTAAGAGCCGCGAGCGATCCTTGAAGCTGTCCCTGATGGTCGTCATCTACCTGCCTGGACAGCATGGCCTGCAACGCGGGCATCCCG

NruI

14193 ATGCCGCCGGAAGCGAGAAGAATCATAATGGGGAAGGCCATCCAGCCTCGCGTCGCGAACGCCAGCAAGACGTAGCCCAGCGCGTCGGCCGCCATGCCCTGCTTCA

14299 TCCCCGTGGCCCGTTGCTCGCGTTTGCTGGCGGTGTCCCCGGAAGAAATATATTTGCATGTCTTTAGTTCTATGATGACACAAACCCCGCCCAGCGTCTTGTCATT

14405 GGCGAATTCGAACACGCAGATGCAGTCGGGGCGGCGCGGTCCCAGGTCCACTTCGCATATTAAGGTGACGCGTGTGGCCTCGAACACCGAGCGACCCTGCAGCGAC

14511 CCGCTTAACAGCGTCAACAGCGTGCCGCAGATCCCGGGCAATGAGATATGAAAAAGCCTGAACTCACCGCGACGTCTGTCGAGAAGTTTCTGATCGAAAAGTTCGA

14617 CAGCGTCTCCGACCTGATGCAGCTCTCGGAGGGCGAAGAATCTCGTGCTTTCAGCTTCGATGTAGGAGGGCGTGGATATGTCCTGCGGGTAAATAGCTGCGCCGAT

14723 GGTTTCTACAAAGATCGTTAGTGGATCGGCACTTTGCATCGGCCGCGCTCCCGATTCCGGAAGTGCTTGACATTGGGGAATTCAGCGAGAGCCTGACCTATTGC

PvuI

14829 ATCTCCCGCCGTGCACAGGGTGTCACGTTGCAAGACCTGCCTGAAACCGAACTGCCCGCTGTTCTGCAGCCGGTCGCGGAGGCCATGGATGCGATCGCTGCGGCCG

14935 ATCTTAGCCAGACGAGCGGGTTCGGCCCATTCGGACCGCAAGGAATCGGTCAATACACTACATGGCGTGATTTCATATGCGCGATTGCTGATCCCCATGTGTATCA

FIG. 13E

15041 CTGGCAAACTGTGATGGACGACACCGTCAGTGCGTCCGTCGCCGCAGGCTCTCGATGAGCTGATGCTTTGGGCCGAGGACTGCCCCCGAAGTCCGGCACCTCGTGCAC

15147 GCGGATTTCGGCTCCAACAATGTCCTGACGGACAATGGCCGCATAACAGCGGTCATTGACTGGAGCGAGGCGATGTTCGGGGATTCCCAATACGAGGTCGCCAACA

15253 TCTTCTTCTGGAGGCCGTGGTTGGCGGGTATGGAGCAGCAGACGCGCTACTTCGAGCGGAGGCATCCGGAGCTTGCAGGATCGCCGCGGCTCCGGGCGTATATGCT

15359 CCGCATTGGTCTTGACCAACTCTATCAGAGCTTGGTTGACGGCAATTTCGATGATGCAGCTTGGGCGCAGGGTCGATGCGACGCAATCGTCCGATCCGGAGCCGGG

15465 ACTGTCGGGCGTACACAAATCGCCCGCAGAAGCGCGGCCGTCTGGACCGATGGCTGTGTAGAAGTACTCGCCGATAGTGGAAACGGGAGATGGGGGAGGCTAACTG

15571 AAACACGGAAGGAGACAATACCGGAAGGAACCCGCGCTATGACGGCAATAAAAAGACAGAATAAAACGCACGGGTGTTGGGTCGTTTGTTCATAAACGCGGGGTTC

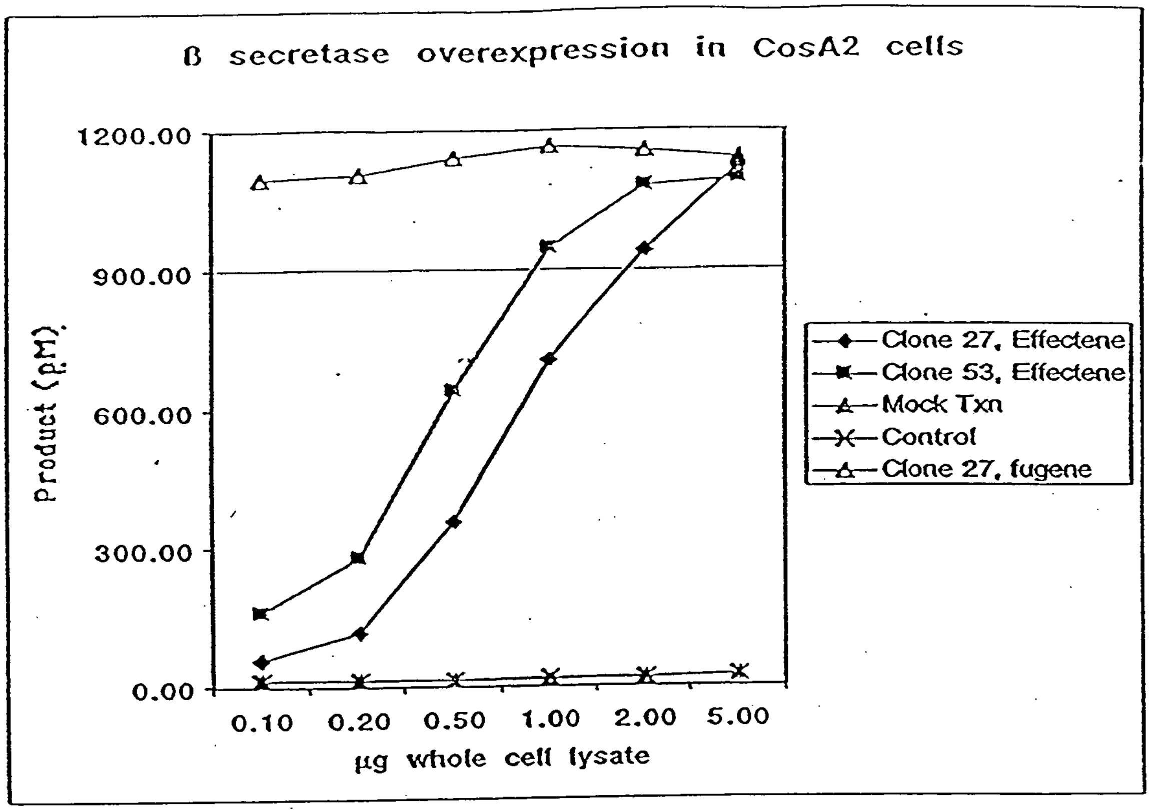
15677 GGTCCCAGGGCTGGCACTCTGTCGATACCCCACCGAGACCCCATTGGGGCCAATACGCCCGCGTTTCTTCCTTTTCCCCACCCCACCCCCCAAGTTCGGGTGAAGG

15783 CCCAGGGCTCGCAGCCAACGTCGGGGCGGCAGGCCCTGCCATAGCCACTGGCCCCCGTGGGTTAGGGACGGGGTCCCCCATGGGAATGGTTTATGGTTCGTGGGG

15889 TTATTATTTTGGGCGTTGCGTGGGGTCTGGTCCACGACTGGACTGAGCAGACAGACCCATGGTTTTTGGATGGCCTGGGCATGGACCGCATGTACTGGCGCGACAC

SalI

15995 GAACACCGGGGCGTCTGTGGCTGCCAAACACCCCCGACCCCCAAAAACCACCGCGCGGATTTCTGGCGTGCCAAGCTAGTCGACCAA



ß secretase overexpression in CosA2 cells
1200.00
900.00
600.00
300.00
0.00
Product (pM)
0.10
0.20
0.50
1.00
2.00
5.00
µg whole cell lysate
Clone 27, Effectene
Clone 53, Effectene
Mock Txn
Control
Clone 27, fugene

FIG. 14

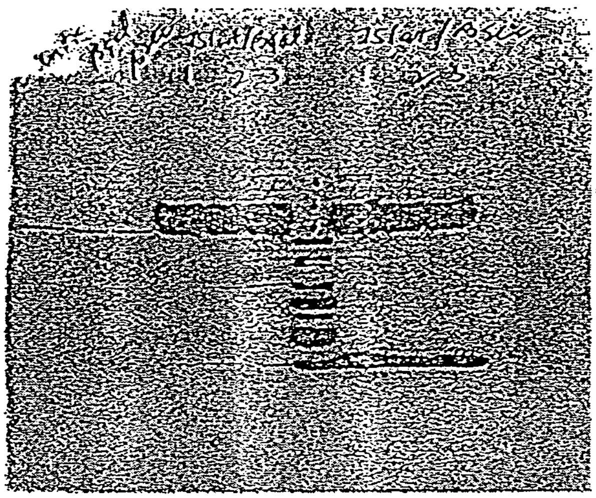


FIG. 15A

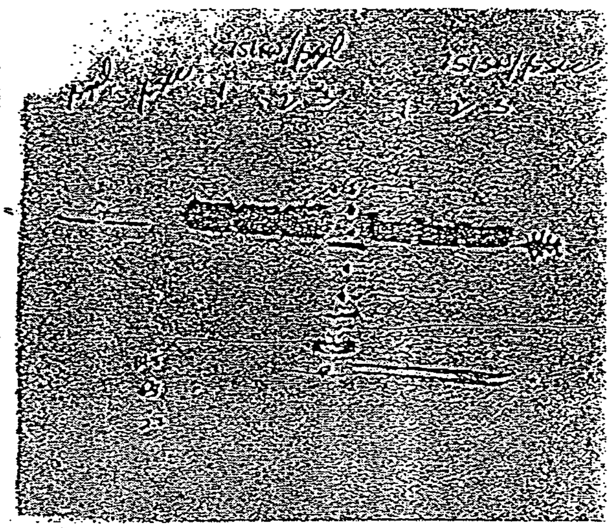


FIG. 15B

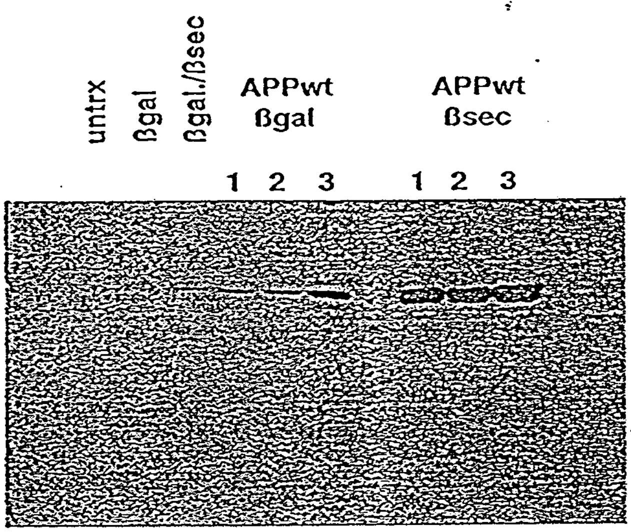


FIG. 16A

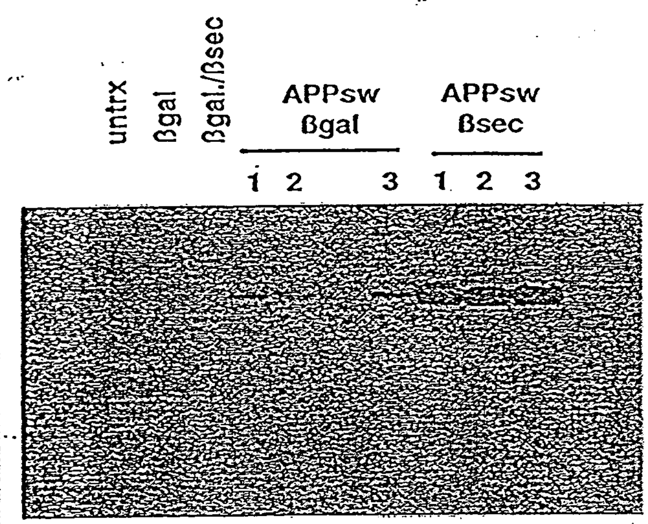


FIG. 16B

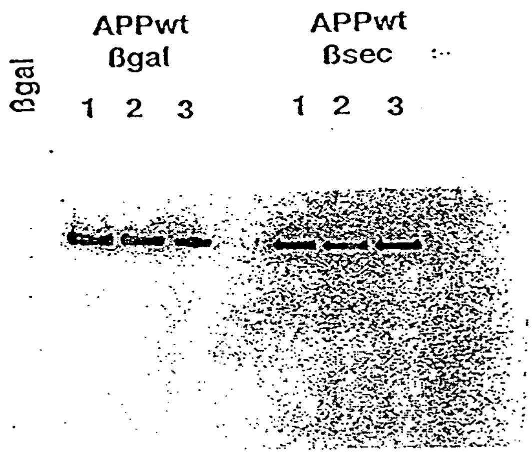


FIG. 17A

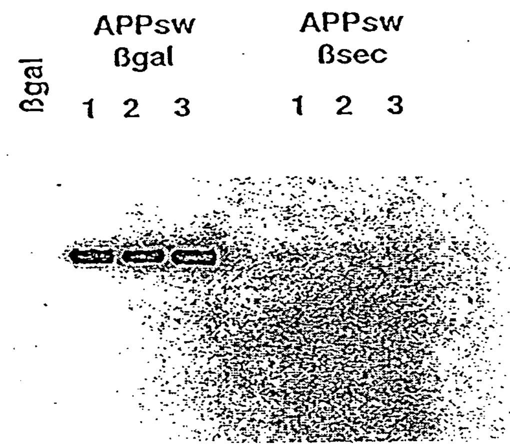


FIG. 17B

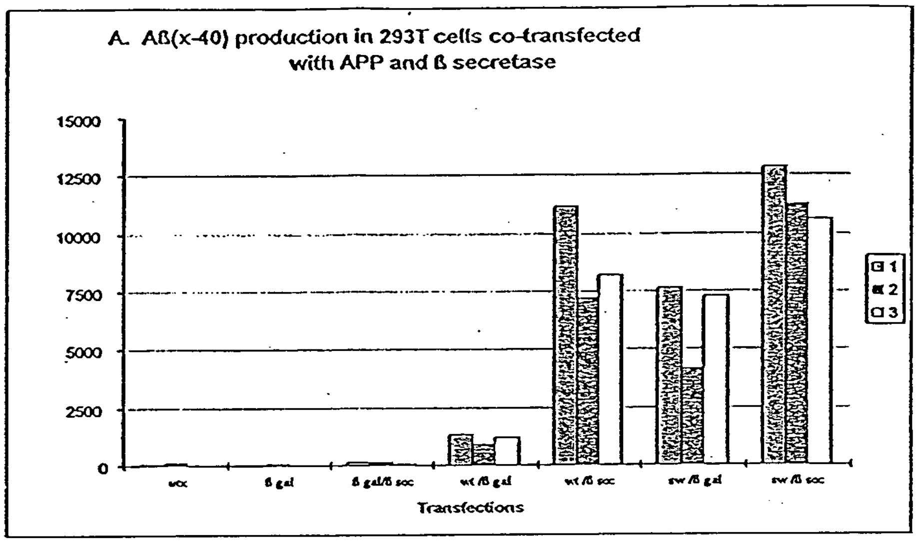

A. Aß(x-40) production in 293T cells co-transfected with APP and ß secretase
15000
12500
10000
7500
5000
2500
0
ß gal
ß gal/ß sec
wt /ß gal
wt /ß sec
sw /ß gal
sw /ß sec
Transfections
1
2
3

FIG. 18

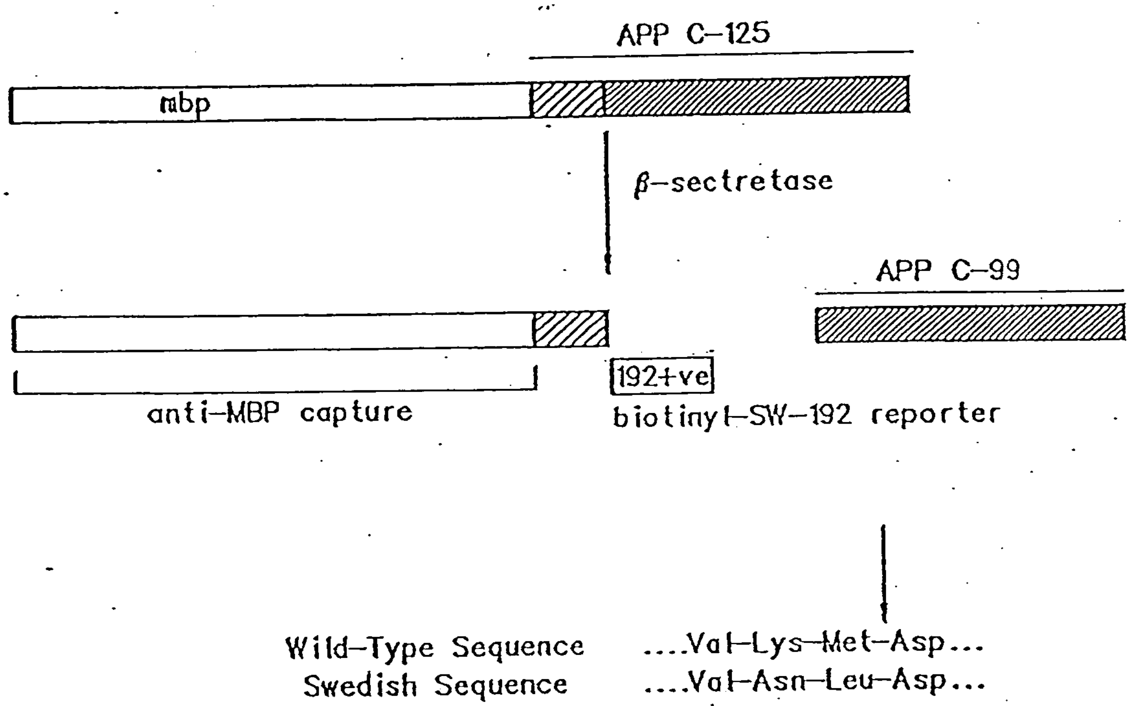
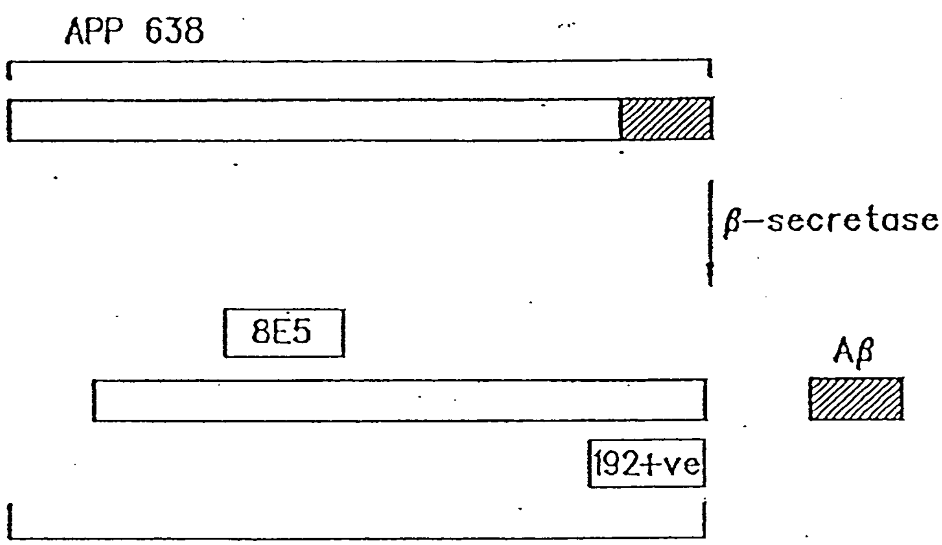

APP C-125
mbp
β-sectretase
APP C-99
192+ve
anti-MBP capture
biotinyl-SW-192 reporter
Wild-Type Sequence ....Val-Lys-Met-Asp...
Swedish Sequence ....Val-Asn-Leu-Asp...

FIG. 19



Detected by: 1. SW192 Western Blot
2. 8E5–192 ELISA

Wild–Type Sequence	....Val–Lys–Met–Asp...
Swedish Sequence	....Val–Asn–Leu–Asp...

FIG. 20

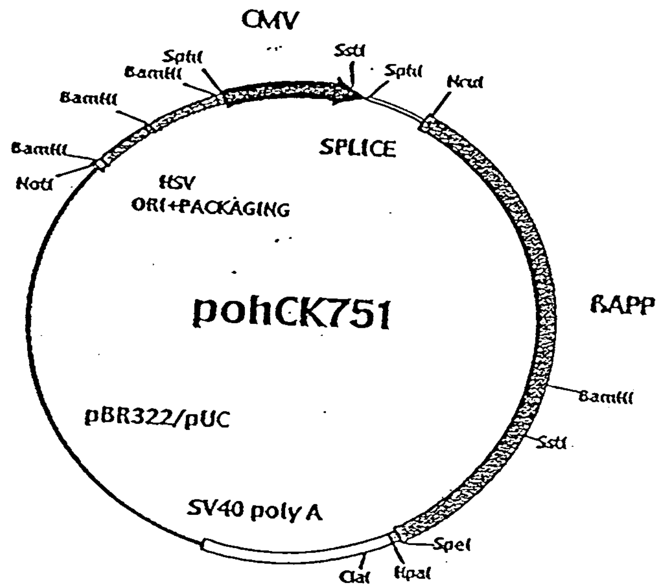

CMV
SstI
SphI
SphI
BamHI
BamHI
NruI
BamHI
NotI
SPLICE
HSV
ORI+PACKAGING
pohCK751
βAPP
BamHI
SstI
pBR322/pUC
SV40 poly A
SpeI
ClaI
HpaI

FIG. 21